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PRESERVATION OF RELICS FROM THE RNA WORLD THROUGH NATURAL SELECTION, SYMBIOSIS AND HORIZONTAL GENE TRANSFER

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ABSTRACT

A difficulty arises if we conjecture that the RNA world may have left molecular relics that may still be extant. We discuss the possible roles that natural selection, symbiosis and horizontal gene transfer may have played in the evolutionary pathway of the earliest RNA replicators. The period that concerns us ranges from the time that separates the appearance of contemporary metazoans at the onset of the Phanerozoic, in the Paleozoic era (the Cambrian period, some 540 million years before the present Mybp), from the RNA world, namely in the Early Archean, or even earlier.

In order to address this question we extend a previous treatment of this problem to non-pathogenic RNA replicators. The possibility of preservation of relics from the RNA world had been discussed earlier in the context of pathogenic RNA replicators (viroids). We discuss the evolutionary forces that may have been at play in the preservation of life from the RNA world to the present. We conjecture that the genes of the replicases (RNA-directed RNA polymerases) associated with putative non-pathogenic DNA-independent replicators of the RNA world may have been transferred vertically, eventually being preserved in some multicellular organisms as RNA replicators independent of DNA. We comment on the outstanding experimental questions that remain to be done, so as to test with new experiments the concept of the preservation of the RNA world relics.

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1. INTRODUCTION

In spite of the success of the concept of the RNA world, which constitutes a "milestone in the scientific study of life's origins" (Joyce and Orgel, 1993), there remains the difficulty of explaining how self-replicating RNA was created during chemical evolution. One of the typical difficulties that remains to be overcome is to be able to provide a complete set of reactions yielding ribose, a key constituent of RNA, as the main product.

An alternative approach to elucidate this important early stage in the evolution of life on earth, is to investigate the question of whether the RNA world may have left any relics amongst pathogens of contemporary multicellular organisms. We discussed one such pathogen (viroids) earlier (Chela-Flores, 1994). We now attempt to answer the question of whether the theory of evolution is compatible with the concept that some cellular non-pathogenic RNA macromolecules may be indicators of the continuity of life, from the earliest stages of its evolution to the present.

A first example of cellular non-pathogenic RNA macromolecules has been reported in the cytoplasm of mitochondria of healthy maize cells (Finnegan and Brown, 1989), consisting of two molecules of lengths 2850 base pairs (bps) and 900 bps. This episomal system ("RNA plasmid") is synthesized in a DNA-independent way.

A second line of research has reported a variety of cellular RNA-dependent RNA polymerases (RdRPase) of no known function. RdRPases are assumed to be the replicases responsible for the replication of molecular relics. RdRPases have been reported to be present in healthy cabbage, cowpea, cucumber, and tobacco (Levings and Brown, 1989).

Non-pathogeneic RNA molecules of unknown origin and unknown gene products may be present in some contemporary cells: double-stranded RNAs have been isolated from leaves of a variety of tobacco (Fraenkel-Conrat, 1983, 1986; Ikegami and Fraenkel-Conrat, 1978, 1979). These RNAs are coded by either one of two of the three genomes of the cell (either the mitochondrion or the nucleus).

2. EVOLUTIONARY FORCES

In the present work we discuss the evolutionary aspects that may support the concept of the preservation of RNA-world relics. In particular we appeal to two well-established evolutionary forces:

a) Natural selection is the principle postulated by Darwin, by means of which variation is preserved, provided it is useful. Natural selection is responsible for the emergence of the present complex phylogenetic relationships amongst the biota, out of which we must identify the particular pathways which may have been followed in the vertical transfer of the early RNA replicators.

b) The Serial Endosymbiosis Hypothesis (Margulis, 1993) gives the evolutionary bases for the structure of the eukaryotic cell. This evolutionary force rationalizes the presence of both the mitochondrion in the metazoan eukaryotic cell. It also rationalizes the presence of the mitochondrion and the chloroplast in metaphytes. Symbiosis may further account for the organelles other than the mitochondrion and the chloroplast (Douglas *et al.*, 1991): In certain algae, such as, cryptomonad flagellates these organelles are called 'nucleomorphs', which probably represent the remains of symbiotic eukaryotes. The process itself by means of which symbiosis succeeds to provide the cell with organelles other than mitochondria or chloroplasts is referred to as 'secondary symbiosis'.

In addition, we also appeal to a possible further evolutionary force:

c) A third factor in evolution is emerging from the growing number of data bank sequences (Smith *et al.*, 1992; Amabile-Cuevas and Chicurel, 1993) that may contribute to our discussion of the relic's pathway. In fact, direct acquisition of the genetic patrimony of foreign species by an organism is called horizontal gene transfer (HGT). Symbiosis represents an example of a massive HGT involving a whole endosymbiotic organism, but HGT may occur independent of the incorporation of new organelles, by either primary or secondary symbioses.

There is as yet no direct evidence for the mechanism that implements the genetic transfer from the organelles to the nucleus (and viceversa). There is, however, ample evidence that HGT occurs extensively in all the kingdoms and also between kingdoms (this aspect of the problem has been recently reviewed in Chela-Flores, 1995). Yet, since in higher plants only transcribed sequences have been found in more than one subcellular compartment, this has suggested that interorganellar transfer of genetic information may occur via RNA; hence, by means of reverse transcriptase the exogenous RNA may be integrated in the genome (Schuster and Brennicke, 1987).

3. POSSIBLE EVOLUTIONARY PATHWAY FOR RNA REPLICATORS

During evolution HGT may have played a role of continually restricting the habitat of the early RNA replicators: First of all, the replicators may have been imported into a cyanobacterium. Subsequently, during the Proterozoic, the RNA replicators may have been imported into an early purple bacterium by bacterial-bacterial conjugation. Mitochondria may have arisen by the invasion of aerobic or anaerobic photosynthetic bacteria into ancestral prokaryotic cells (Kuntzel and Kochel, 1981). Later, at an advanced stage of protistan diversification, algae emerged close to the metazoa-metaphyte radiation.

As we shall discuss below, some of the spore-fossil evidence supports the conjecture that land plants may have emerged in the Ordovician from charophycean algae. It is

generally agreed that the angiosperms descended from the progymnosperms, whose first appearance goes back to at least 370 Mybp. Finally, it is plausible that the monocot-dicot divergence may have occurred some 200 Mybp (Wolfe *et al.*, 1989).

We introduce the following hypothesis: In the ancestral lineage of the metaphytes the DNA-independent RNA replicators may have persisted in both prokaryotic and eukaryotic cells as non-pathogenic relics by the combined effect of repeated symbiosis and HGT. The validity of this hypothesis is discussed for putative RNA replicators in the following section with some emphasis on the evolutionary factors involved.

4. DNA-INDEPENDENT RNA REPLICATORS MAY BE MOLECULAR RELICS

4.1 Are there RdRPases in cyanobacteria and purpule bacteria?

It has been shown in the various experiments shown in Sec. 1 that RNA plasmids are specific to some plant cells. We recall that according to the Serial Endosymbiosis Theory some chloroplasts originated through polyphyletic associations between various cyanobacteria and the precursors of eukaryotic cells.

A property of RNA plasmids - replication due to a host enzyme - suggests that a cyanobacterium RNA replicase should be searched for (i.e., an RdRPase), since such an enzyme would be responsible for the replication of the DNA-independent RNA replicators.

Specificity should also be studied in the various classes of cyanobacteria: Coccogoneae and Hormogoneae. These taxa should also be considered from this point of view, since there are many examples of laminated stromatolites that were constructed by filamentous organisms. It should also be remarked that there are examples of laminated fossil stromatolites, where the constructing organisms apparently were coccoid rather than filamentous (Walter, 1983)

Finally, specificity of the RNA replicators should also be tested in members of Chloroxybacteria, since this class includes the putative cyanobacterium genus ancestral to chloroplasts *(Prochloron)*

4.2 On the possibility of vertical transfer of genes

The case of *Cyanophora paradoxa* is particularly useful as an illustration in the present context of the potential role of HGT. *C. paradoxa* is a flagellated protist, an euglenoid harboring cyanelles, which are cyanobacterium-like symbionts lacking cell walls. Cyanelles are functional chloroplasts and are known to have only 10% the DNA content of a nonsymbiotic cyanobacterium (Maynard-Smith, 1991). These endosymbionts have many proteins coded by nuclear genes. After symbiosis the symbiont may have a much smaller genome, being constrained to

rely on its host's genomic repertoire, as in the above well-studied case of the cyanelles.

What is already known about the integration of the cyanelle genomes with their hosts may be a model of what to expect in our present case of interest:

Cyanobacteria, being the putative hosts of DNA-independent RNA replicators first became chloroplasts of plant cells at some early stage in evolution, the most likely precursor genus ancestral to chloroplasts being *Prochloron*. In this case the cyanobacterium becomes a *de facto* organelle. In so doing, the chloroplast may lose the ability of its precursor (i.e., the cyanobacterium) to code for the enzyme RdRPase.

The proposed work remaining in plant biology is not an easy task for a number of reasons: It is evident that bryophytes and tracheophytes arose from green algae, and that the particular ancestral group was similar to extant Charophyta (Lewis, 1991).

Some support for this view is that flavonoid compounds, such as anthocyanin, are probably present in all tracheophytes, but are mostly absent from seedless vascular plants. Anthocyanin is a particularly significant biochemical indicator of angiosperms. This is underlined by the observation that in the process of expressing anthocyanin in a dicot, a specific transcriptional activator from a monocot is able to increase the dicot-anthocyanin biosynthesis. In this example, the monocot is maize *(Zea mays* L.*)* and the dicots are members of the subclass Dilleniidae *(Arabidopsis)* and of the subclass Asteridae *(Nicotiana)* (Lloyd *et al.,* 1992).

In this context it is interesting to remark that some flavonoid compounds are present in charophycean green algae (Swain, 1991). This supports the hypothesis that this group of seaweeds may have been ancestral to land plants.

On the other hand, chloroplasts may have evolved from photosynthetic bacteria more than once. This is a consequence of considering the distribution and comparison of chlorophylls. Grass green plastids (chlorophyll a and b) indicate that they were acquired from organisms of the *Prochloron* group of bacteria, whereas blue-green and red plastids probably derived from another class of cyanobacterium (Coccogoneae, coccoid cyanobacteria). This, however, does not alter our hypothesis.

4.3 A possible pathway of RNA replicators from the RNA world to the metaphytes.

RNA replicators at an early stage in evolution may have been specific to *Prochloron.* According to the above argument some members of this cyanobacterium genus later became organelles of higher eukaryotes, probably of charophycean green algae that led to the origin of land plants. One consequence of these comments is that specificity of the RNA replicators should be studied in *Coleochaete*. This is the extant genus of

charophycean green algae which, as already emphasized, most closely resembles the now extinct ancestors of the land plants. This option may be interpreted as a second experiment that suggests itself, namely, testing the vertical transfer of the RdRPase gene in charophycean green algae.

Some additional difficulties underlie the series of events that may have led from the first prokaryotes (in the Archean) to single-cell eukaryotes (in the Proterozoic) and, finally, to multi-cellular organisms (in the Phanerozoic).

In agreement with our hypothesis, the genes codifying for the proposed RdRPase, in a given cell must have been transferred vertically in a series of symbioses during evolution:

While algae may have first appeared during the Riphean period late in the Proterozoic era, the oldest definitive evidence of tracheophytes (except for Ordovician fossilized spore tetrads) is from the Late Silurian, some 410 Mybp (Gray and Shear, 1992).

Progymnosperms (intermediate plants between nonseed-bearing and seed-bearing tracheophytes) were present during the Upper Devonian, and pteridosperms (seed-bearing plants with fern-like foliage) were present in Permian and Carboniferous flora. The angiosperms themselves may have arisen from Bennettitales, an extinct group related to living cycads. At each of the underlying successive symbiotic events in the evolution of plants, from its photosynthetic prokaryotic ancestors, plastids must have shared genes with their hosts (in principle even the gene coding for the putative RdRPase).

5. DISCUSSION AND CONCLUSIONS

5.1 How can we put on more solid basis the conjecture that relics of the RNA world may be specific to some metaphytes?

The gene coding for the putative RdRPase may have been horizontally transferred in successive vertical transfers that may have occurred during plant evolution from late in the Proterozoic era (algae) to the Lower Cretaceous period (monocots and dicots).

In this manner non-pathogenic DNA-independent RNA replicators may have persisted in each of the various tracheophytic taxa in the lineage of the most advanced metaphytes, namely monocots and dicots. As discussed above, there is some evidence for RNA replicators apparently not directly related to the DNA-based replication of the cell.

To sum up, RNA replicator specificity should be tested in different plant, protist, and prokaryotic taxa that are in the lineage ancestral to the angiosperms.

If successful, the proposed extensive experimental work discussed in this paper would remove the difficulty implied by the rather recent time of the first appearance of the taxon in

which the putative relics are suggested to exist. The preservation of the relics of the primordial RNA world may have followed a possible pathway from cyanobacteria and purple bacteria to charophycean green algae and, finally, to the tracheophytes.

In other words, before the many experiments suggested above are completed, we cannot exclude the possibility that DNA-independent RNA replicators could have had a continuous pathway from the RNA world to the present.

5.2 Paleontological evidence for the earliest organisms.

The earliest date assigned to fossils of the Archean is about 3,500 Mybp (Schopf, 1993); the micropaleontological work has been done in the Warrawoona Group (part of the Lower Archean Pilbara Supergroup) in northwestern Western Australia. These microbes resemble cyanobacteria.

This microfossil-containing environment has produced fossils in organosedimentary domes known as stromatolites. These rocks consist of regular laminated mats built up by microbial communities, which are mainly cyanobacteria. If these most ancient prokaryotes may be shown to be capable of being associated with non-pathogenic DNA-independent RNA replicators, and this property may be further shown to be possible in various taxa in the lineage of the higher tracheophytic taxa, then we would be able to interpret the data in terms of RNA relics that may have been present during the major part of the duration of life on earth (Chela-Flores, 1994).

We have discussed in the present work the roles that symbiosis and HGTs may have played beyond natural selection, which during the vertical gene transfer provided the non-pathogenic RNA replicators a diverse set of cellular hosts, which have been well adapted to the severe environmental changes that such a long geological time span has produced on the evolution of the earth itself.

5.3 Some difficulties from the paleontological evidence for establishing the complete pathway for the RNA-world relics

We should remark that extant sedimentary rocks have been retrieved from the Isua peninsula in western Greenland with dates prior to the earliest cyanobacteria from the Pilbara Supergroup but, unfortunately, these ancient samples are severely altered by metamorphism, thus barring any certain inferences from paleobiological techniques.

One possibility has recently been formulated for bridging this fundamental gap in our understanding of the preservation of molecular relics from the RNA world (Arrhenius *et al.*, 1993). Apatites (phosphates, arsenates and vanadates) are very common minerals, stable in many environments. These minerals can form directly from within a sediment, and are called authigenic. In deposits formed from solutions with pH less than 8.5, authigenic sedimentary apatite appears to be a

reliable indicator of live organisms, whose presence can be established, even in the absence of fossils.

Such an approach may eventually provide an independent chemical indicator of the earliest appearance of life on earth for the all-important period preceding the the date assigned to the earliest stromatolites. These novel viewpoints may yet overcome the barrier to the information that is possible to retrieve from the Archean rocks which have been subjected to metamorphism. An example of such rocks are those from the Isua peninsula in West Greenland (3,800 Mybp). The same difficulty regarding metamorphism would apply to the rocks from the Acasta gneiss in North America, which have been assigned a date of 3,960 Mybp.

However, the isotopic signature of the Isua rocks may already be an indicator that life may have been present at this early stage in the evolution of the earth (Schildowski, 1988). In this case, it may be possible from paleontological evidence to support most of the evolutionary pathway for the early RNA replicators, which we have suggested in the present work.

On the other hand, the isotopic signature may raise some difficulties concerning its validity as an indicator of early photosynthetic organisms. For instance, the evidence coming from geochronology may lead to revise the dates assigned to the earliest rocks available at present (cf., Moorbath, 1995). If this point of view is taken into consideration, then we should have to rely on conceptual improvements, in order to complete the pathways for the RNA-world relics. This could be achieved by means of work such as that based on the use of phosphate, which we have mentioned above.

5.4 Conclusion

We still have not reached a position in which we could attempt to trace the *complete* evolutionary pathways of the early replicators of the RNA world. Our current understanding is incomplete with regards to the molecular genetics of the organisms in the lineage of the higher taxa of the tracheophytes. The bases for this uncertainty is the existence of a comparatively small (in a geologic scale) gap in the knowledge in the evolution of life that can be based on paleontological evidence. Nevertheless, it is still possible to set upper and lower bounds to this gap:

An upper bound may be inferred from the date assigned to the earliest stromatolites. On the other hand, from what is known from lunar exploration we can obtain an estimate on the earliest possible time when the RNA world could have existed. We should recall that according to the evidence gathered by the Apollo missions, the end of the period of heavy bombardment by meteorites may have occurred at about 4,000 Mybp. Prior to this time no form of life was possible. This sets a lower bound to the gap in our knowlege of the continuous existence of life on earth from the earliest stages of its evolution.

Future progress in molecular biology, micropaleontology, geochemistry and geochronology may yet offer us an opportunity for extending the present work. It should eventually be possible to provide a complete scenario on the preservation of RNA replicators from the primordial RNA world to the present.

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